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## Improving the quality of soybeans by alkaline microwave treatment

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**Abstract.** The article aimed to investigate the effect of combined microwave and alkaline treatment on the nutritional value of soybeans and the degree of inactivation of trypsin inhibitors. Experimental studies were conducted on soybeans of Ukrainian origin with an initial moisture content of 9%. The buffer capacity of soybeans was determined and it was found that to achieve pH 8, it is necessary to add 1% baking soda by weight of the raw material. Three soybean fractions

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were studied: whole beans, coarse grinding (2-3 mm) and fine grinding (0.5 mm). It was found that the coarsely ground fraction demonstrated the optimal ratio between moisture retention capacity and alkaline solution permeability. Microwave treatment was carried out at a power of 600 W for 30-150 seconds with a step of 30 seconds. To assess the qualitative parameters, an infrared spectrometer was used to determine the activity of trypsin inhibitors and protein solubility in potassium hydroxide. The results showed that the combined microwave-alkali treatment provided more effective inactivation of trypsin inhibitors compared to pure microwave treatment. At a treatment time of 150 seconds, the activity of trypsin inhibitors decreased from 29.8 mg/g in the control sample to 7.1 mg/g in the microwave treatment and to 4.7 mg/g in the combined microwave-alkaline treatment, which is a decrease of 76.2% and 84.2%, respectively. At the same time, the protein solubility remained at a high level – 87.4% for microwave and 88.2% for microwave-alkaline treatment. It has been established that a preliminary increase in pH to 8 contributes to the partial destruction of the buffer properties of protein components and increases the efficiency of subsequent heat treatment. The optimal processing parameters were determined as raw material moistening up to 15%, use of coarsely ground fraction, preliminary alkaline treatment to pH 8, and microwave heating for 120-150 seconds at a power of 600 W. The proposed method allows to significantly increase the nutritional value of soy raw materials while maintaining high protein solubility, which makes it promising for implementation in the food industry and feed production

**Keywords:** trypsin inhibitors; antinutritional factors; protein denaturation and solubility; alkaline and heat treatment

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## Introduction

Soybean represents one of the most valuable sources of plant protein in global agriculture and the food industry. However, its widespread application is constrained by the presence of antinutritional factors, amongst which trypsin inhibitors and haemagglutinins occupy a leading position, reducing protein digestibility and the biological value of soy products. Soybeans account for approximately 25% of global oil-seed production and serve as a key component in the food industry and livestock farming (Turpurova & Kurbatov, 2024). The high protein content (35-40%) and balanced amino acid composition render soybean an indispensable ingredient in the manufacture of food products, compound feeds, and protein concentrates (Kostyna & Bronnikova, 2024). Nevertheless, according to G. Padalkar *et al.* (2023), the full utilisation of soybean protein is limited by the presence of antinutritional factors,

the most significant of which are trypsin inhibitors and haemagglutinins. Trypsin inhibitors account for 5-7% of the total protein content of raw soybeans and are of two main types: Kunitz inhibitor and Bowman-Birk inhibitor. These compounds block the activity of proteolytic enzymes in the digestive tract, reducing protein digestibility and amino acid bioavailability. Long-term consumption of raw soybeans causes pancreatic hypertrophy, reduced growth in animals, and impaired nutrient absorption (Liu, 2024). Therefore, inactivation of antinutritional factors is a critically important step in preparing soybean raw materials for use.

As stated by B. Iegorov *et al.* (2023), traditional heat treatment methods, in particular autoclaving and extrusion, although they provide sufficient inactivation of trypsin inhibitors, have significant disadvantages, including high energy consumption, process duration,

and the risk of denaturation of a significant number of protein molecules with loss of their functional properties. In this regard, it is urgent to search for alternative processing methods that would combine high efficiency of inactivation of antinutrients with preservation of the nutritional value of proteins. Among modern approaches, the use of microwave heating is of particular interest. This method provides rapid and volumetric heating of the raw material due to dielectric heating, which occurs as a result of the interaction of an electromagnetic field with polar water molecules. Unlike convection heating, where heat is transferred from the surface to the centre, microwave heating provides a more uniform temperature distribution throughout the product, which contributes to more effective inactivation of anti-nutritional factors with lower energy consumption (Xiang *et al.*, 2020).

An additional promising direction is the combination of thermal and chemical treatment by regulating the acid-base balance of the medium. According to Z. Luo *et al.* (2025), alkaline treatment promotes protein denaturation and the breaking of disulphide bonds in the structure of trypsin inhibitors, which increases their sensitivity to thermal inactivation. At the same time, an alkaline environment can improve the solubility of proteins and their functional properties, which is an important factor for further technological use. Despite the existence of separate studies on the effectiveness of microwave processing and alkaline modification of soy protein, a comprehensive approach that would combine both methods, taking into account the optimisation of processing parameters (fractional composition of raw materials, moisture, pH level, duration and power of microwave heating), remains insufficiently studied (Sui *et al.*, 2021; Das *et al.*, 2024). Therefore, the purpose of this study was to determine the optimal parameters of combined microwave-alkali treatment of soybeans for maximum inactivation of trypsin inhibitors while maintaining

high protein solubility and nutritional value of the product. To achieve the goal, it was necessary to solve the following tasks: (1) to investigate the effect of moisture and fractional composition of soybeans on the degree of inactivation of trypsin inhibitors during microwave treatment; (2) to establish the buffer capacity of soybeans and calculate the required amount of baking soda to achieve the optimal pH level for preliminary alkaline treatment; (3) to assess the effect of microwave treatment duration on the activity of trypsin inhibitors and protein solubility in samples moistened with distilled water and treated with an alkaline solution.

### Literature Review

The problem of inactivation of anti-nutritional factors in legumes has been studied for several decades, and a significant amount of scientific data has been accumulated on the effectiveness of various processing methods. Traditional approaches are based on thermal denaturation of protein inhibitors; however, the intensity and duration of treatment significantly affect not only the inactivation of antinutrients, but also the preservation of the nutritional properties of the final product. The structure of soybean trypsin inhibitors is characterised by the presence of disulphide bonds, which ensure the stability of their three-dimensional conformation. The Kunitz inhibitor contains two disulphide bridges, while the Bowman-Birk inhibitor contains seven such bonds, which makes the latter more heat-resistant (Park *et al.*, 2023). Inactivation of these compounds under the influence of high temperature occurs through the destruction of disulphide bonds and denaturation of the protein structure, which leads to the loss of their inhibitory activity.

Studies by J. Gu *et al.* (2022) showed that complete inactivation of trypsin inhibitors in raw soybeans requires prolonged boiling: 14 minutes provides 80% inactivation, and 30 minutes provides about 90% inactivation. However, such prolonged thermal exposures

can negatively affect protein quality, reducing its solubility and bioavailability of amino acids. Therefore, it is urgent to find methods that would ensure effective inactivation at lower temperature-time parameters. Microwave heating demonstrates significant advantages over convection methods due to the dielectric heating mechanism. Studies by I. Munro *et al.* (2003) confirmed that microwave treatment effectively reduces trypsin inhibitor activity and improves protein digestibility: microwaving soy milk at 100°C for 8 minutes increased protein digestibility by 7% compared to traditional heating, which requires 30 minutes to achieve the same effect. A critical factor in the effectiveness of microwave processing is the moisture content of the raw material. Studies of lipoxygenase inactivation in soybeans have shown that at a moisture content of 26.9-56.8%, complete inactivation of the enzyme is achieved after 210 seconds of microwave heating (Cao *et al.*, 2023). Moisture acts as a dielectric medium, ensuring uniform distribution of microwave field energy throughout the product volume and contributing to more efficient heating of the internal layers. At the same time, excessive moisture reduction during microwave processing can lead to uneven heating and local overheating. Studies by Z. Luo *et al.* (2025) have shown that microwave heating is more effective in changing the oil distribution in soybean seeds compared to autoclaving, but the degree of protein denaturation remains lower. This highlights the need to optimise raw material moisture to achieve a balance between the efficiency of inactivation of antinutritional factors and the preservation of protein quality.

The particle size of the soybean raw material significantly affects the speed and uniformity of heating. Whole beans are characterised by a slow heating rate due to low thermal conductivity and the presence of a water-impermeable shell. Grinding increases the surface area and improves heat access to the endosperm, but excessively fine fractions

can quickly lose moisture during microwave heating, reducing processing efficiency. Studies show that pre-soaking soybeans before microwave processing provides a higher level of trypsin inhibitor inactivation compared to dry raw materials (Munro *et al.*, 2003). This is explained by the increase in humidity, which promotes uniform heating, and partial swelling of protein structures, which makes them more accessible for thermal denaturation. However, the duration of the soaking process and subsequent drying may be technologically impractical in industrial conditions.

Adjusting the pH of the medium is a powerful tool for modifying the functional properties of soy protein. Alkaline treatment leads to the destruction of the spatial configuration of protein molecules due to the electrostatic repulsion of negatively charged groups and the destruction of hydrogen bonds. Studies of pH-shifting technology have shown that processing soy protein isolate at pH 12 followed by neutralisation to pH 7 significantly improves solubility, surface hydrophobicity, and emulsifying properties (Tang & Ma, 2009). It is important to note that alkaline treatment (pH 12 → 7) has a more pronounced effect on the structure and functional properties of the protein compared to acid treatment (pH 2 → 7), since the change in protein structure in an alkaline environment is more intense, according to L. Zheng *et al.* (2022). This is confirmed by an increase in the content of free thiol groups by 13-19% and an increase in surface hydrophobicity by 59-61% after heating at pH 12 (Chen *et al.*, 2013).

Combined alkaline and heat treatment demonstrates a synergistic effect. Raising the pH to 9 followed by heating provides a greater increase in protein solubility compared to the separate application of alkaline or heat treatment (Wu *et al.*, 2009). This occurs due to the partial destruction of the buffering properties of protein components and the facilitation of thermal denaturation of trypsin inhibitors. The Institute of Feed and Agriculture of Podillia of

the National Academy of Agricultural Sciences of Ukraine has developed a method for eliminating anti-nutritional factors in soybeans by treating crushed grain in a 2.5% calcium hydroxide solution (Vysochanska & Petrychenko, 2010). The technology involves soaking soybean flour in water (ratio 1:4) with the addition of 25 g/kg of alkali to achieve pH 7. The use of this method allows to reduce the content of antinutrients and improve the digestibility of proteins, but requires a long processing time and subsequent washing of the raw materials.

A generalisation of the literature data shows that the most promising is an integrated approach that combines the advantages of various processing methods. The combination of microwave heating with pre-moistening and pH adjustment can provide a synergistic effect in inactivating anti-nutritional factors at lower energy costs and shorter processing times. However, most studies focus on the separate study of the effect of microwave treatment or alkaline modification, while the comprehensive optimisation of processing parameters (fractional composition, moisture, pH level, microwave heating modes) remains poorly studied. In addition, it is important to determine the optimal balance between the degree of inactivation of trypsin inhibitors and the preservation of the functional properties of the protein, in particular its solubility, which is a critical indicator for the technological use of soy raw materials. Thus, the relevance of this study lies in the development of a scientifically sound approach to combined microwave-alkaline processing of soybeans with the determination of optimal parameters that ensure maximum inactivation of trypsin inhibitors while maintaining high protein solubility and nutritional value of the product.

## Materials and Methods

Characteristics of raw materials and reagents. For experimental research, soybeans of the 'Sigaliya' variety from the 2024 harvest in Chernihiv

region (Ukraine) were used. The research was conducted in the spring of 2025. The raw materials were stored in paper bags at a temperature of 18-20°C and a relative humidity of 30-40%. The initial moisture content of the beans was 9%, which corresponds to standard storage conditions for oilseeds (DSTU 4964:2008, 2010). The crude protein content in the control samples was 33.7%, and crude fat was 21.5%. Baking soda (sodium bicarbonate,  $\text{NaHCO}_3$ ) with a mass fraction of the basic substance of at least 99.9% was used as an alkaline reagent. The choice of soda as an alkaline agent was due to its wide application in the food industry, safety of use, and the ability to effectively regulate the pH level without introducing toxic components or heavy metals. Distilled water with a specific electrical conductivity of no more than 5  $\mu\text{S}/\text{cm}$  was used to prepare the solutions.

Determination of the buffering capacity of soybeans. The buffer capacity of soybeans was determined titrimetrically using a standardised 0.1 M baking soda solution. A 5.00 g portion of crushed soybeans (0.5 mm fraction) was placed in a 100 ml beaker and 50 ml of distilled water was added. The suspension was left for 20 minutes at room temperature ( $22 \pm 2^\circ\text{C}$ ) for swelling of protein structures and hydration of colloidal components, stirring periodically with a glass rod. After swelling, 0.1 M baking soda solution was gradually added to the suspension in small portions of 0.1-0.2 ml using an automatic pipette, mixing thoroughly after each addition for 30 seconds. One minute after adding the next portion of alkali, the pH of the suspension was measured using universal indicator litmus strips with a pH determination range of 5-9 (determination accuracy  $\pm 0.2$  pH units) manufactured by Lachema (Czech Republic). The initial pH of the aqueous suspension of soybeans was  $6.3 \pm 0.1$ . The volume of soda solution required to increase the pH by one unit was recorded to construct a titration curve and calculate the buffer capacity. The experiment was repeated five times to ensure statistical reliability of the

results. The buffer capacity ( $\beta$ ) was calculated using the formula (1):

$$\beta = \frac{\Delta C}{\Delta \text{pH}}, \quad (1)$$

where  $\Delta C$  – change in the concentration of added alkali (mol/l);  $\Delta \text{pH}$  – change in the pH of the suspension.

Preparation of samples of different fractions. To determine the optimal particle size of soybean raw materials, three types of samples were prepared: (1) whole beans – soybeans without grinding, previously cleaned of damaged and defective seeds by manual sorting; (2) coarse grinding (2-3 mm) – beans were ground in a laboratory mill OlisLab 2100 (a modernised version of the LZM-1 mill, LLC “Olis”, Ukraine), the resulting fraction was sieved through a set of sieves with a mesh size of 2.0 and 3.0 mm to standardise particle size; (3) fine grinding (0.5 mm) – beans were ground using a laboratory mill Retsch ZM-300 (Makrolab LTD, Ukraine) at a rotation speed of 14,000 rpm, using a 0.5 mm ring sieve.

Humidification of raw materials. A control group of samples weighing 100 g each was moistened with distilled water until a humidity of 15% was reached. The volume of water required for moistening was calculated using the formula (2):

$$V = \frac{m \times (W_2 - W_1)}{100 - W_2}, \quad (2)$$

where  $m$  – sample mass (g);  $W_1$  – initial humidity (%);  $W_2$  – final moisture content (%). For a 100 g sample with an initial moisture content of 9%, the required volume of distilled water was 7.06 ml. Water was added to the samples evenly using a micro-spray sprayer with constant stirring to ensure uniform moisture distribution. After humidification, the samples were placed in sealed polyethylene bags and kept for 60 minutes at room temperature to evenly redistribute moisture throughout the volume of the raw material. The actual moisture content of the samples after exposure was controlled by

the gravimetric method by drying at a temperature of  $105 \pm 2^\circ\text{C}$  to constant weight.

Alkaline treatment. The experimental group of samples was treated with a solution of baking soda to achieve a pH of 8. Based on the data from the determination of the buffer capacity, it was established that to achieve this pH level, it is necessary to add 1.0% of soda by weight of the raw material (1.0 g per 100 g of soybeans). The soda was dissolved in the calculated volume of distilled water (7.06 ml). The resulting alkaline solution was used to moisten soybean samples similarly to the control group using a spray gun. After moistening, the samples were kept in sealed bags for 60 minutes to evenly distribute the alkali and establish an equilibrium pH value. pH control was performed on aqueous extracts (sample: water ratio = 1:10) 60 minutes after treatment. The actual pH value of the extracts was  $7.9 \pm 0.1$ , confirming that the target alkalinity level was achieved.

Microwave processing. Microwave treatment was performed in a household microwave oven Grunhelm 20MX711-B (Ukraine) with a nominal power of 800 W. To ensure the same processing conditions for all samples, the 600 W mode (75% power) was used, which provides more controlled heating conditions and reduces the risk of local overheating. Samples weighing 100 g were placed in plastic containers and distributed in a uniform layer 8-10 mm thick to ensure uniform heating. The containers were placed in the centre of the rotating plate of a microwave oven. The duration of treatment varied from 30 to 150 seconds in 30-second increments, which allowed for assessing the kinetics of trypsin inhibitor inactivation and changes in protein solubility depending on the exposure time. After microwave treatment, the samples were cooled to room temperature in air for 30 minutes, after which the mass loss due to moisture evaporation was determined. The treated samples were ground to a fraction of 0.5 mm and stored in sealed plastic containers at a temperature of

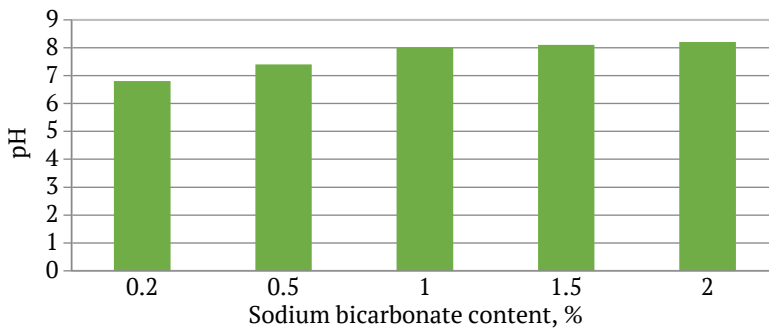
$4 \pm 2^\circ\text{C}$  until analytical studies were performed, but not more than 24 hours.

**Methods of analysis.** To assess the quality indicators of soybean samples, a Bruker TANGO infrared spectrometer (Germany) with Evonik calibration was used, which provides rapid and non-destructive determination of quality indicators. The device operates in the near-infrared spectrum (850-1,050 nm) and uses chemometric models to calculate component concentrations based on spectral characteristics. Determination of trypsin inhibitor activity and protein solubility in potassium hydroxide (KOH) was performed by the spectrometric method using a calibration model developed for soy products. The results were expressed in milligrams of trypsin inhibitors per gram of dry matter (mg/g) and as a percentage of total protein, respectively. The relative error of determination was no more than 1%. Five parallel samples were prepared for each treatment

option, which ensured high statistical reliability of the results. Statistical processing of the obtained results was carried out according to V. Matviychuk *et al.* (2021) using variance and correlation analysis ( $p=0.05$ ).

## Results and Discussion

The dependence of the pH change on the amount of added alkali was established by titrating an aqueous suspension of crushed soybeans with a solution of baking soda (Fig. 1). The initial pH value of the suspension was  $6.3 \pm 0.1$ , which is consistent with the data of X. Xiong *et al.* (2015) on the weakly acidic reaction of aqueous soybean extracts. Adding 0.2% soda by weight of the raw material increased the pH to 6.8, at 0.5% – to 7.4, and at 1.0% – to 8.0. Further increase in the soda concentration to 1.5% and 2.0% did not cause significant changes in the pH (8.1 and 8.2, respectively), which indicates that a buffer plateau has been reached.



**Figure 1.** Change in pH depending on the amount of sodium bicarbonate

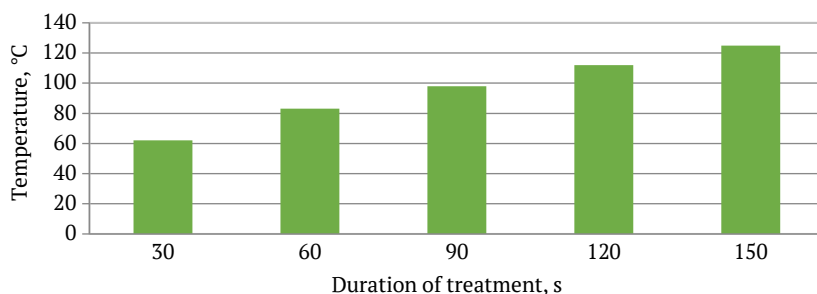
**Source:** compiled by the authors

The established buffer curve demonstrated the typical behaviour of protein systems with high buffer capacity in the pH range of 6-8. This was due to the presence of monoaminocarboxylic amino acids in the composition of soy proteins, in particular glutamic and aspartic acids. The buffer capacity of soybeans in the pH range of 6.3-8.0 was  $0.18 \pm 0.02$  mol/( $l \times \text{pH unit}$ ), which is a relatively high indicator and confirms the ability of soy protein to resist

pH changes. For practical use, the optimal concentration of soda was chosen to be 1.0% of the mass of the raw material, which ensured the achievement of pH 8.0 – a level sufficient for partial destruction of the buffer properties of the protein without excessive alkalinity, which can lead to undesirable chemical modifications of amino acids. A similar approach was used in pH-shifting technology, where pH 8-9 was considered optimal for improving

the functional properties of soy protein (Wu *et al.*, 2009). Comparative analysis of samples with an initial moisture content of 9% and moistened to a level of 15% revealed the critical role of moisture content in the efficiency of microwave heating. At a microwave power of 600 W, the temperature in the centre of the

moistened samples after 30 seconds of treatment was  $62 \pm 3^\circ\text{C}$ , after 60 seconds –  $83 \pm 4^\circ\text{C}$ , seconds –  $98 \pm 5^\circ\text{C}$ , seconds –  $112 \pm 6^\circ\text{C}$ , and at 150 seconds it reached  $125 \pm 7^\circ\text{C}$  (Fig. 2). The coefficient of temperature variation at different points of the sample did not exceed 8%, which indicates a fairly uniform heating.



**Figure 2.** Temperature change in the centre of humidified samples depending on the duration of treatment

**Source:** compiled by the authors

In contrast to the moistened samples, dry beans (9% moisture) exhibited significant heating non-uniformity with temperature variations ranging from  $70^\circ\text{C}$  to  $150^\circ\text{C}$  within a single sample. The coefficient of variation of temperature was 25-35%, indicating that heating was localised mainly in the outer layers of the sample. Visual inspection revealed partial burning of the surface layer in the form of dark brown spots, while the internal parts remained insufficiently heated. This is consistent with the data by H. Cao *et al.* (2023) on the critical role of moisture as a dielectric mediator in microwave heating. Water molecules, having a high dipole moment, effectively absorb the energy of the electromagnetic field and convert it into heat. At low humidity, heating occurs less intensively, and local areas with higher moisture content overheat, which leads to uneven processing. Increasing humidity to 15% provides a more uniform distribution of dielectric heating throughout the product volume.

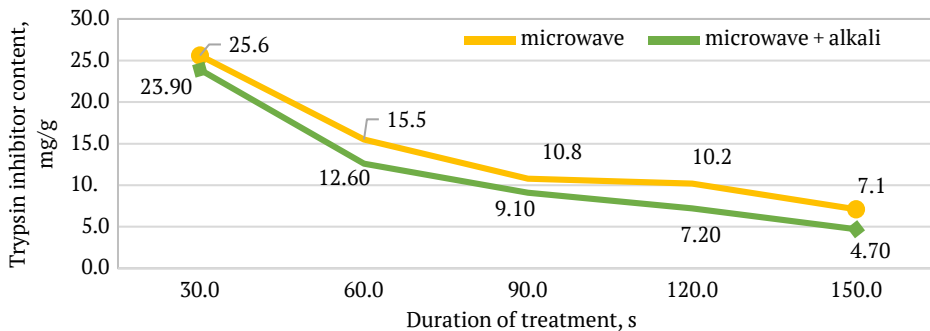
The study of the effect of particle size on the efficiency of combined processing revealed

significant differences between the three fractions studied. Whole soybeans were characterised by uniform heating and good moisture retention during microwave processing. The weight loss after 150 seconds of heating was only  $3.2 \pm 0.4\%$ , indicating effective water retention in the bean structure. However, the activity of trypsin inhibitors after alkaline treatment followed by microwave heating for 150 seconds decreased only to  $18.3 \pm 1.2$  mg/g compared to  $29.8$  mg/g in the control (a decrease of 38.6%). The low efficiency of alkaline treatment of whole beans is explained by the presence of a waterproof shell, which limits the penetration of the alkaline solution to the endosperm, where the bulk of proteins and trypsin inhibitors are concentrated. The structure of the soybean shell includes a cuticle with a waxy layer and an epidermis with palisade cells, which form a barrier to the diffusion of aqueous solutions (Ma *et al.*, 2004). For effective alkaline treatment of whole beans, a long pre-soaking (8-12 hours) is required, which is technologically impractical.

The fine fraction (0.5 mm) exhibited the opposite problem – excessively rapid moisture loss during microwave heating. The mass loss after 150 seconds was  $11.8 \pm 1.5\%$ , with intensive evaporation occurring after 60 seconds of treatment. Visual inspection showed the formation of a dry crust on the surface of the sample, which was accompanied by a sharp increase in temperature to  $140\text{--}160^\circ\text{C}$  in some areas. The activity of trypsin inhibitors during this treatment was almost unchanged. The reason for the low efficiency is the early evaporation of moisture, which occurs before the temperature sufficient to denature the protein inhibitors is reached. Studies by J. Gu *et al.* (2022) show that effective inactivation of trypsin inhibitors requires a temperature of  $90\text{--}100^\circ\text{C}$  and a sufficient duration of exposure. During rapid drying of the fine fraction, the temperature rises to  $140\text{--}160^\circ\text{C}$ , but due to the lack of moisture, heat transfer to the internal particles deteriorates, which reduces the efficiency of denaturation.

The coarse grinding fraction (2–3 mm) was found to be optimal, providing a balance between moisture retention capacity and accessibility

to alkaline solution. Mass loss after 150 seconds of microwave treatment was  $5.8 \pm 0.6\%$ , indicating moderate moisture evaporation with sufficient moisture retention for effective heat transfer. It was this fraction that provided the best results in inactivation of trypsin inhibitors during combined treatment. Grinding to a size of 2–3 mm destroys the bean shell and increases the surface area of the endosperm, which facilitates the penetration of the alkaline solution and provides better contact with the protein components. At the same time, particles of this size retain sufficient structural integrity to retain moisture during microwave heating. This is consistent with the data by H. Vagadia *et al.* (2017) on the positive effect of pre-grinding on the efficiency of thermal processing of legumes. The results of determining the activity of trypsin inhibitors in samples after different treatment options are presented in Figure 3. The control sample without treatment was characterised by a trypsin inhibitor activity of  $29.8 \pm 1.2$  mg/g, which is a typical value for raw soybeans and is consistent with the data of M. Friedman & D. Brandon (2001).



**Figure 3.** Effect of duration of treatment duration and type on the activity of trypsin inhibitors  
**Source:** compiled by the authors

In microwave-only processes, a gradual decrease in the activity of trypsin inhibitors was observed with increasing heating duration (Fig. 3). The transition between a short interval of 30 seconds provided a decrease in activity to 25.6 mg/g (by 14.1% compared to

the control), i.e. to the level of reaching a temperature of  $62^\circ\text{C}$  – the level of the beginning of denaturation of the most heat-sensitive protein fractions. The most intense decrease in activity occurs between 30–60 seconds (from 25.6 to 15.5 mg/g), when the sample

temperature is 83°C and denaturation of the Kunitz inhibitor occurs, which is less heat-stable compared to the Bowman-Birk inhibitor, according to A. Park *et al.* (2023). Subsequently, with an increase in the treatment duration to 90-120 seconds, the rate of decrease in the activity of trypsin inhibitors decreased (change from 15.5 to 10.2 mg/g), which indicates a higher thermal stability of the Bowman-Birk inhibitor with seven disulphide bonds and requires a longer thermal exposure for complete denaturation (Kumar *et al.*, 2006). During treatment for 150 seconds (125°C), the activity decreased to only 7.1 mg/g, corresponding to a 76.2% reduction compared to the control.

Optimal microwave-alkaline treatment demonstrated significantly higher efficiency of inactivation of trypsin inhibitors at all studied time ranges. Already after treatment for 30 seconds, the activity decreased to 23.9 mg/g, which was 6.6% less compared to pure microwave treatment for the same duration. The most pronounced effect was observed in the case of treatment for 120-150 seconds, when the combined treatment provided a reduction in activity to 7.2 and 4.7 mg/g, respectively, which was 29.4% and 33.8% more effective compared to microwave treatment alone. At 150 seconds duration, the combined treatment reduced the activity of trypsin inhibitors by 84.2% to a level of 4.7 mg/g, which corresponds to the limit level considered safe for use in feed purposes (<5 mg/g), according to P. Mittal *et al.* (2021). This confirms the synergistic effect of the combination of alkaline and heat treatment. Increasing the pH to level 8 leads to partial ionisation of carboxyl and amino groups of protein molecules, which leads to electrostatic repulsion and spatial change of the tertiary structure of the protein, as mentioned by C. Tang & C. Ma (2009) and M. Zhong *et al.* (2024). This makes disulphide bonds more accessible for hydrolysis under the influence of high temperature and accelerates the rate of denaturation of trypsin inhibitors. Correlation

analysis showed a high negative correlation between the duration of treatment and the activity of trypsin inhibitors both during microwave treatment ( $r = -0.986$ ,  $p < 0.001$ ) and during combined treatment ( $r = -0.992$ ,  $p < 0.001$ ), which confirms the pattern of inactivation of antinutritional factors with increasing thermal exposure time.

The experiments conducted confirm the literature data on the effectiveness of microwave treatment for inactivating trypsin inhibitors. Experimental results obtained by I. Munro *et al.* (2003) confirmed that microwave heating of soy milk at 100°C for 8 minutes reduced the activity of trypsin inhibitors by 70-75%, which is comparable to the observations of the present research with a processing time of 150 seconds. At the same time, the use of combined alkaline-thermal treatment provides the same or greater effect in a shorter duration, which is of practical importance in optimising the energy efficiency of the technological process. It is worth noting that even with the longest processing time (150 seconds), protein solubility remained at a fairly high level – 87.4% for microwave and 88.2% for microwave + alkali. This indicates a relatively mild nature of the thermal effect, which ensures effective inactivation of trypsin inhibitors without excessive denaturation of protein structures, which is important for preserving the technological properties of soy raw materials.

Analysis of the kinetics of inactivation of trypsin inhibitors indicated the most intense decrease in their activity in the range of 30-90 seconds of treatment when the temperature of the samples increased from 60°C to 100°C. This corresponds to the denaturation temperature of the main soy proteins:  $\beta$ -conglycinin (75-80°C) and glycinin (85-95°C). In this temperature range, protein globules unfold and aggregation processes develop, which is accompanied by the destruction of active sites of trypsin inhibitors. With a treatment duration of more than 120 seconds, the rate of inactivation slows

down, which may be due to several factors. First, by this point, inactivation of the most thermolabile fraction of inhibitors has already occurred, and the predominantly thermostable Bowman-Birk inhibitor remains. Secondly, at temperatures above 110°C, intensive evaporation of moisture begins, which reduces the efficiency of heat transfer and can lead to local overheating instead of uniform heating. From a practical point of view, the most optimal time for combined microwave-alkaline treatment can be considered to be the interval of 120-150 seconds, when the activity of trypsin inhibitors is 4.7-7.2 mg/g while maintaining protein solubility at 87.8-88.2%. This indicator is close to the results of soybean meal processed by traditional methods (autoclaving, extrusion), where the residual activity of trypsin inhibitors is usually 3-5 mg/g.

Modern soybean processing technologies include autoclaving (121°C, 15-20 minutes), extrusion (130-150°C, 30-60 seconds), and toaster roasting (110-120°C, 20-30 minutes). Autoclaving provides complete inactivation of trypsin inhibitors, but requires a long processing time and significant energy consumption. Extrusion is more energy efficient, but requires special equipment and can lead to excessive protein denaturation with a loss of solubility of up to 70-75%. The proposed method of combined microwave-alkali treatment has several advantages. First, the treatment time is only 120-150 seconds, which is significantly shorter than autoclaving (15-20 minutes) or frying (20-30 minutes). Secondly, the energy consumption is quite low: at a power of 600 W and a duration of 150 seconds, the energy consumption is about 0.025 kWh per 100 g of raw material, which is a competitive indicator. Thirdly, the preservation of high protein solubility (88.2%) makes the processed raw material suitable for a wide range of technological applications. An additional advantage is the possibility of continuous processing in industrial conditions. Conveyor-type microwave

plants allow for a continuous process with precise control of processing parameters, which is more difficult for batch autoclave plants. Alkaline pre-wetting can be integrated into the process line as a preparation stage before microwave heating, which does not require additional equipment and does not significantly complicate the process.

Based on the research performed, the following technological recommendations can be formulated for combined microwave-alkali processing of soybean raw materials. Raw material preparation: use soybeans with an initial moisture content of 8-10%, cleaned of impurities and damaged seeds. Grind to a coarse fraction with a particle size of 2-3 mm, which provides an optimal balance between accessibility to alkaline solution and the ability to retain moisture during microwave heating. Alkaline treatment: moisten the crushed raw materials with a solution of baking soda (1% of the mass of the raw materials) until a moisture content of 15% and a pH of  $8.0 \pm 0.2$  are reached. Allow to stand for 60 minutes to evenly distribute the alkali throughout the volume of the raw materials. Monitor the pH of the aqueous extract (ratio 1:10) with indicator strips or a pH meter. Microwave treatment: microwave heating at 600 W for 120-150 seconds. Place the raw materials in an even layer up to 10 mm thick to ensure uniform heating. Monitor the temperature of the samples, which should be 110-125°C at the end of the treatment. Quality control: determine the residual activity of trypsin inhibitors (target value <7 mg/g) and the solubility of protein in KOH (not lower than 85%). If necessary, adjust the processing time depending on the characteristics of the individual batch of raw materials.

## Conclusions

The use of combined microwave-alkali treatment of soybeans is an effective treatment method for the effective inactivation of trypsin inhibitors while maintaining high protein

solubility. It has been established that the coarsely ground fraction (2-3 mm) provides the best balance between moisture retention capacity and accessibility to the alkaline solution, while whole beans are characterised by insufficient penetration of alkali through the waterproof shell, and the fine fraction (0.5 mm) has excessively rapid moisture loss during microwave heating. It has been established that achieving pH 8 is ensured by adding 1.0% baking soda to the mass of raw materials, which contributes to the partial violation of the buffer properties of protein components and increases the efficiency of subsequent heat treatment. The critical role of raw material moisture for the efficiency of microwave processing has been confirmed. Humidification of soybean raw materials to a level of 15% ensured uniform heating with a temperature variation coefficient of no more than 8%, while processing dry beans with a humidity of 9% was characterised by high uneven heating (variation coefficient of 25-35%) with local overheating of the surface layer and insufficient heating of the internal parts.

It was found that combined microwave-alkaline treatment provides more effective inactivation of trypsin inhibitors compared to pure microwave treatment at all studied time intervals. At a processing time of 150 seconds and a power of 600 W, the activity of trypsin inhibitors decreased from 29.8 mg/g in the control sample to 7.1 mg/g with microwave processing (a 76.2% reduction) and to 4.7 mg/g with combined microwave-alkaline processing (a 84.2% reduction), which is 33.8% more effective compared to pure microwave processing. It has been proven that combined processing provides effective inactivation of anti-nutritional factors while maintaining high protein solubility. Under optimal processing conditions (120-150 seconds), protein solubility remained at 87.8-88.2%, which is sufficient to preserve the technological properties of soy raw materials and its suitability for use in food products and feed. The synergistic effect of combining

alkaline and heat treatment is explained by the partial opening of protein structures in an alkaline environment, which makes the disulphide bonds of trypsin inhibitors more accessible for thermal cleavage, while the violation of the buffer properties of protein components reduces the intensity of denaturation under the influence of temperature. A favourable combined processing regime has been formed: moistening the material to 15%, using a coarsely ground fraction (2-3 mm), preliminary alkaline treatment to  $\text{pH } 8.0 \pm 0.2$  by adding 1% baking soda, microwave heating at a power of 600 W for 120-150 seconds until a temperature of 110-125°C is reached.

The study has certain limitations that must be taken into account when interpreting the results. The experiments were conducted on laboratory equipment with a limited number of samples (100 g). Scaling up to industrial volumes may require correction of processing parameters due to the peculiarities of heat transfer in large volumes of raw materials. Industrial microwave units have a different chamber shape and electromagnetic field distribution system, which may affect the uniformity of heating. Another limitation is that only one pH level (8.0) was investigated during operation. A systematic study of the pH behaviour over a wider range (7.5-9.5) could allow for the establishment of an optimum and the assessment of the sensitivity of the process to changes in this parameter. It is also promising to investigate alternative alkaline agents, such as calcium or potassium hydroxide, which may provide additional technological or nutritional benefits.

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### **Conflict of Interest**

None.

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## **Підвищення якості сої шляхом лужної СВЧ-обробки**

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**Анотація.** Метою статті було дослідити вплив комбінованої мікрохвильової та лужної обробки на поживність сої та ступінь інактивації інгібіторів трипсину. Експериментальні дослідження проводилися на соєвих бобах українського походження з початковою вологістю 9 %. Визначено буферну ємність сої та встановлено, що для досягнення рН 8 необхідно додавання 1 % харчової соди від маси сировини. Досліджено три фракції сої: цілі боби, крупний помел (2-3 мм) та дрібний помел (0,5 мм). Встановлено, що фракція крупного помелу демонструє оптимальне співвідношення між вологоутримувальною здатністю та проникністю лужного розчину. Мікрохвильову обробку проводили при потужності 600 Вт протягом 30-150 секунд з кроком 30 секунд. Для оцінки якісних показників застосовували інфрачервоний спектрометр із визначенням активності інгібіторів трипсину та розчинності білка у гідроксиді калію. Результати показали, що комбінована СВЧ-лужна обробка забезпечує більш ефективну інактивацію інгібіторів трипсину порівняно з чистою СВЧ-обробкою. При тривалості обробки 150 секунд активність інгібіторів трипсину знижувалася з 29,8 мг/г у контрольному зразку до 7,1 мг/г при СВЧ-обробці та до 4,7 мг/г при комбінованій СВЧ-лужній обробці, що становить зниження на 76,2 % та 84,2 % відповідно. При цьому розчинність білка залишалася на високому рівні – 87,4 % для СВЧ та 88,2 % для СВЧ-лужної обробки. Встановлено, що попереднє підвищення рН до 8 сприяє частковому руйнуванню буферних властивостей білкових компонентів та підвищує ефективність подальшої термічної обробки. Оптимальними параметрами обробки визначено зволоження сировини до 15 %, використання фракції крупного помелу,

попередню лужну обробку до рН 8 та СВЧ-нагрівання протягом 120-150 секунд при потужності 600 Вт. Запропонований метод дозволяє значно підвищити поживну цінність соєвої сировини зі збереженням високої розчинності білка, що робить його перспективним для впровадження у харчовій промисловості та кормовиробництві

**Ключові слова:** інгібітори трипсину; антипоживні фактори; денатурація та розчинність білка; лужна та термічна обробка